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## Inhibition of the high-affinity brain glutamate transporter GLAST-1 via direct phosphorylation.

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Conradt M, Stoffel W.

Institute of Biochemistry I, Medical Faculty, University of Cologne, Germany.

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Neurotransmission at excitatory glutamatergic synapses is terminated by the reuptake of the neurotransmitter by high-affinity transporters, which keep the extracellular glutamate concentration below excitotoxic levels. The amino acid sequence of the recently isolated and cloned brain-specific glutamate/aspartate transporter (GLAST-1) of the rat reveals three consensus sequences of putative phosphorylation sites for protein kinase C (PKC). The PKC activator phorbol 12-myristate 13-acetate (PMA) decreased glutamate transport activity in *Xenopus* oocytes and human embryonic kidney cells (HEK293) expressing the cloned GLAST-1 cDNA, within 20 min, to 25% of the initial transport activity. This downregulation was blocked by the PKC inhibitor staurosporine. GLAST-1 transport activity remains unimpaired by phorbol 12-monomyristate. Removal of all putative PKC sites of wild-type GLAST-1 by site-directed mutagenesis did not abolish inhibition of glutamate transport. [32P]Phosphate-labeled wild-type and mutant transport proteins devoid of all predicted PKC sites were detected by immunoprecipitation after stimulation with PMA. Immunoprecipitation of [35S]methionine-labeled transporter molecules indicates a similar stability of phosphorylated and nonphosphorylated GLAST-1 protein. Immunofluorescence staining did not differentiate surface staining of HEK293 cells expressing GLAST-1 with and without PMA treatment. These data suggest that the neurotransmitter transporter activity of GLAST-1 is inhibited by phosphorylation at a non-PKC consensus site.

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